ISOTOPIC FRACTIONATION OF CHROMIUM AND URANIUM DURING CR(VI) REDUCTION BY ASCORBATE AND U(VI) REDUCTION BY SULFIDE

BY

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THESIS

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ABSTRACT

Chromium and uranium naturally occur in a variety of Earth's waters. Both elements can undergo valence state changes at surface conditions which control their chemical behavior in near surface settings. Reduction of hexavalent chromium (Cr(VI)) and uranium (U(VI)) greatly decreases their solubility and mobility, and therefore their toxicity in groundwater. Redox transformations have been shown to induce predictable shifts in isotope ratios for both elements. As a result, Cr and U isotope ratios can be used to track the extent of reduction independent of problems related to dilution, advection, and adsorption that plague the standard concentration-based approach.

The magnitude of isotopic fractionation has been determined for various abiotic and biotic reduction reactions in the Cr and U systems; other reductants of interest remain to be studied. Using batch reactor experiments, this study quantifies the magnitude of isotopic fractionation associated with reduction of Cr(VI) to Cr(III) by ascorbate and reduction of U(VI) to U(IV) by sulfide.

The results of this study yielded isotopic fractionation factor values (ε) for reduction of Cr(VI) by ascorbate of -2.83‰ ±0.05‰ and -3.16‰ ±0.23‰ in two duplicate experiments. These results are closely similar to earlier experiments using organic reductants. In contrast, reduction of U(VI) via sulfide does not induce significant isotopic fractionation. The $^{238}U/^{235}U$ in the remaining U(VI) appears to have increased by 0.17‰ after about 60% reduction, but the analytical uncertainty was about 0.15‰.

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INTRODUCTION

The presence of chromium (Cr) and uranium (U) in the environment poses risks to human health via land, surface water, and groundwater contaminations. Elevated Cr concentrations arise from natural processes like weathering of ultramafic rocks (e.g., Robles-Camacho and Armienta, 2000) and industrial processes such as metal plating, leather tanning, corrosion prevention, wood preservation, and pigment manufacturing (Nriagu and Nieboer, 1988; US EPA, 1998). Likewise, elevated U concentrations originate from natural weathering of rocks, from waste materials related to the mining, extraction, and manufacturing of nuclear fuel and weapons, and from fly ash generated by coal combustion (Benes, 1999; Ferraiolo et al., 1990). Negative health effects on human health of Cr(VI) and U(VI) include development of respiratory tract infections, ulcers, anemia, and lung cancer (US EPA, 1998) via exposure by inhalation, ingestion, or direct contact (Benes, 1999). Cr(VI) has severe human health effects due to its carcinogenic and mutagenic properties (Losi et al, 1994; De Flora, 2000). Additionally, bioavailable Cr and U affects the health of wildlife populations (Markich, 2002).

Better understanding of Earth's paleoredox condition through time, particularly those related to fluctuations of oxygen abundance of the atmosphere and oceans, is a major goal in geochemistry. Paleredox indicators provide insight into drivers of the evolution of life over time, ancient atmospheric chemistry, and, because of the connection between O_2 and the carbon cycle, possible insight into the future consequences of human impact (Brennecka et al., 2011a; Frei et al., 2009; Lyons et al., 2009; Weyer et al., 2008). However, extracting information about past redox conditions from rocks can be difficult, and new geochemical tools are sought to help in this regard.

In aqueous solutions, Cr and U have similar chemical behaviors, controlled by their valence states. Cr is a transition metal compatible in Earth's mantle. It is generally found in high concentrations (~1000 ppm) in mantle-derived mafic and ultramafic rocks (Izbicki et al., 2008). At surface conditions, Cr is thermodynamically stable in the hexavalent (Cr(VI)) or trivalent (Cr(III)) valence state. Under oxidizing conditions at circum-neutral pH, hexavalent Cr(VI) is soluble and dominates as toxic chromate (CrO_4^{2-}) and hydrochromate ($HCrO_4^-$) anions. Under reducing conditions, Cr(VI) reduces to Cr(III), an insoluble, less toxic species.

U is a naturally occurring radioactive actinide, thermodynamically stable in the hexavalent (U(VI)) or tetravalent (U(IV)) valence state in groundwater systems. Hexavalent U(VI) dominates in oxidizing conditions, forming uranyl (UO_2^{2+}) cations, which in turn form strong soluble, mobile, anionic complexes with carbonate, phosphate, and organic ligands (Murphy and Shock, 1999). Under reducing conditions and near-neutral pH, U(VI) reduces to U(IV), an insoluble species, decreasing U mobility and thus, toxicity in groundwater.

Redox transformations are a fundamental process in Cr and U cycling throughout geologic history and across many geochemical settings. Cr and U reduction can occur by biological mechanisms (e.g., microbes living in aquifers) and by abiotic reaction with reductants such as organic acids and minerals like FeS, siderite, magnetite, or green rust (e.g., Pettine et al., 1998; Viamajala et al., 2002; Morales et al., 2007; Asatiani et al., 2004; O'Loughlin et al., 2003, Missana et al., 2003; Hua and Deng, 2008; Ithurbide et al. 2009, Du et al., 2011). Reduction of Cr and U from their hexavalent states significantly decreases their solubility and mobility in groundwater. If reduction products are stable, then reduction may provide a favorable long-term remediation method for contaminated groundwater (Blowes, 2002).

Because Cr and U reduction are so important in environmental management decisions, detecting and possibly quantifying extent of reduction is highly desirable. However, doing this using concentration measurements can be difficult, time-consuming, and expensive. A measured decrease in Cr or U concentration in an aquifer can result from one or more of several processes, including redox transformation, dilution, transport, adsorption, or coprecipitation. To determine the presence and extent of Cr or U reduction, it must be distinguished from these other processes.

Isotope methods are an encouraging alternative for monitoring and quantifying Cr(VI) and U(VI) reduction (Johnson and Bullen, 2004; Ellis et al., 2002; Bopp et al., 2009). Cr has four naturally occurring stable isotopes: ^{50}Cr, ^{52}Cr, ^{53}Cr, and ^{54}Cr with abundances 4.35%, 83.79%, 9.50%, and 2.37%, respectively. All U isotopes are radioactive. Isotopes of U with half-lives longer than 100,000 years are ^{233}U, ^{234}U, ^{235}U, ^{236}U and ^{238}U. Of these, ^{235}U (0.72% abundance; $t_{1/2} \sim 7.038 \times 10^8$ yrs) and ^{238}U (99.28% abundance; $t_{1/2} \sim 4.468 \times 10^9$ yrs) are the two isotopes of interest due to their relatively high natural abundances (Weyer et al., 2008). Because their half-lives are so long, decay is insignificant during the time scales relevant to modern environmental studies, and these isotopes can be effectively treated as stable isotopes.

Slight differences in bond energies between isotopes of an element arise from differences in the masses and, in very heavy elements, the nuclear volumes of isotopes. These differences cause particular isotopes to react preferentially compared to others. The lower zero point energy (ZPE) of heavier isotopes results in their preferential reaction (Ellis et al., 2002). Through this kinetic isotope fractionation, the product of Cr(VI) reduction, Cr(III), is always enriched in lighter Cr isotopes than the reactant pool. As a result of the preferential removal of lighter isotopes, the remaining unreacted Cr(VI) becomes enriched in heavier isotopes, and this enrichment increases predictably as reduction proceeds (Ellis et al., 2002).

In contrast, U isotope fractionation has been observed to occur in a reversed sense. Reduction of U(VI) preferentially consumes ^{238}U at a slightly greater rate relative to ^{235}U, leaving the reactant pool enriched in ^{235}U and the solid U(IV) product enriched in ^{238}U (Basu et al., 2014; Bopp et al., 2010; Montoya-Pino et al., 2010; Weyer et al., 2008; Brennecka et al. 2011). This reverse sense of fractionation probably arises from a phenomenon known as the nuclear volume effect (Schauble, 2007; Bigeleisen, 1996). Theoretical studies of equilibrium isotopic fractionation between U(VI) and U(IV) show that mass dependent fractionation is small and is overwhelmed by fractionation related to differing volumes of the nuclei of different isotopes (Bigeleisen, 1996). Based on theoretical considerations of this "nuclear volume effect," it had been predicted that for very heavy elements like Hg and U, isotopes with larger nuclei are thermodynamically more stable when they are found in states with lower electron density at the nucleus (Abe et al., 2008; Schauble, 2007). The reduced U(IV) species has lower electron density at the nucleus relative to U(VI) because its two additional electrons are in 5f or 6d orbitals that "screen" s-electrons (Schauble, 2007). Therefore, at isotopic equilibrium, U(IV) is enriched in the heavy ^{238}U relative to U(VI) (Abe et al, 2008). Kinetic isotope effects apparently follow suit, with the U(IV) produced by U(VI) reduction being enriched in ^{238}U (Stirling et al., 2007; Weyer et al., 2008; Bopp et al., 2009, Basu et al., 2014). The resulting isotopic fractionation can be quantified via measurements of the $^{238}U/^{235}U$ ratio. Because $^{238}U/^{235}U$ varies in response to redox reactions, it should be useful as a redox indicator for both modern and ancient environments.

Current research indicates that isotopic fractionation is much greater for redox transformations than for other processes, making isotope ratio measurement variations a unique indicator for redox reactions. Chemical processes that do not change the valence of Cr or U

involve much smaller bonding changes and thus processes like adsorption do not induce strong isotopic fractionation (Ellis et al., 2004; Brennecka, 2011b).

Quantification of isotopic fractionation in Cr and U is done via measurement of the isotope ratios $^{53}Cr/_{52}Cr$ and $^{238}U/_{235}U$. Because variations in the isotopic ratios are so small (usually less than 1%), $\delta^{53}Cr$ and $\delta^{238}U$ notation is used. This notation describes the parts-per-thousand (‰) deviation of an isotope ratio relative to a standard:

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000‰ \qquad (1)$$

where R_{sample} and $R_{standard}$ are the isotope ratios of the sample and standard, respectively (e.g., $^{53}Cr/_{52}Cr$, $^{238}U/_{235}U$).

The magnitude of isotopic fractionation is described using the instantaneous fractionation factor, α:

$$\alpha = \frac{R_{product}}{R_{reactant}} \qquad (2)$$

where $R_{product}$ and $R_{reactant}$ are the isotope ratios for the reaction product flux and reactant pool at any time point during the reaction.

For convenience, α is converted to a per mil parameter, ε:

$$\varepsilon = 1000‰ \times (\alpha - 1) \qquad (3)$$

which is convenient because it is a very close approximation to the difference in δ values between the reactant and product flux:

$$\varepsilon \sim \delta_{reactant} - \delta_{product} \qquad (4)$$

Several experiments determining ε for Cr and U reduction reactions have been performed (Basu and Johnson, 2012; Bopp et al., 2010; Kitchen, et al., 2012; Sikora et al., 2008; Basu et al., 2014; Ellis et al., 2002; Berna et al., 2010), but fractionation factors of some reductants of interest remain to be tested. Cr abiotic experiments show some systematic differences in ε associated with different reduction mechanism or classes of reductants.

This study quantifies the magnitude of isotopic fractionation associated with two reduction reactions. The first reaction studied was reduction of Cr(VI) to Cr(III) by ascorbate ($C_6H_8O_6$). This reaction was chosen for study because the Kitchen et al. (2012) study involved only a few organic molecules as reductants. Ascorbate is known to reduce Cr(VI) (Stearns and Wetterhahn, 1994), and the results given here extend knowledge of the range of isotopic fractionation occurring during Cr(VI) reduction by organic molecules.

The second reaction studied was reduction of U(VI) to U(IV) by dissolved sulfide. This is a potentially important reaction in bioremediation settings, where sulfate reduction can produce sulfide, which in turn may reduce U(VI) (Druhan et al., 2008). Aside from a recent abstract by Stylo et al. (2014), which finds no fractionation for reduction of U(VI) with FeS, little is known about U isotopic fractionation during abiotic U(VI) reduction; this study provides some of the first data to address this issue. Isotopic fractionation factors were determined by carrying out the reduction reactions in controlled batch reactor experiments, and measuring isotope ratios in the remaining dissolved Cr(VI) or U(VI).

METHODS

MATERIALS

Reagent grade potassium dichromate purchased from Fisher Scientific was used as the source of hexavalent chromium. Disodium hydrogen phosphate (Na_2HPO_4) buffer, PIPES buffer, sodium bicarbonate ($NaHCO_3$), l-ascorbic acid ($C_6H_8O_6$ reductant), and Na_2S (reductant) were all ACS reagent grade. High purity (18MΩ-cm) deionized water (Millipore Corp., USA) was used for making all solutions and dilutions.

Hexavalent uranium stock solution was made from uranium metal standard CRM 112-A. Metal was dissolved in concentrated HNO_3, then repeatedly re-dissolved and dried, with each redissolution done in concentrated HNO_3. This uranyl nitrate solution was dried down completely, then twice re-dissolved in concentrated HCl and dried down. Finally, the uranyl chloride solution was completely dried down and re-dissolved in a degassed 100 mM $NaHCO_3$ solution to make a ~4000 mg/L uranyl carbonate stock solution.

EXPERIMENTAL PARAMETERS AND PROCEDURES

Cr(VI) reduction by ascorbate

To determine reaction rate, a preliminary experiment with Cr(VI) reduction by ascorbate was performed using methods similar to the kinetic rate experiments reported by Xu et al. (2004). Before adding the ascorbate reductant, a glass beaker with stir bar contained 100 mL of solution composed of 38.5 μM Cr(VI) and 303 μM Na_2HPO_4. The phosphate buffer maintained pH close to 6.7 throughout the experiment. Reduction commenced with addition of 5.0 mL of 2.27 mM ascorbate, resulting in 115 μM ascorbate in the total experiment volume. Samples were removed periodically to measure Cr(VI) concentration. As in the experiments by Xu et al.

(2004), this experiment demonstrated removal of half the initial Cr(VI) within less than one minute (Fig. 1). This reaction rate was too fast to confidently measure a time series $\delta^{53}Cr$ progression as reduction proceeded because of uncontrolled subsequent reduction after sampling. Accordingly, a multi-step method, described below, was used in which each step proceeded to completion.

Anaerobic batch experiments for isotope ratio measurements were performed in 130 mL glass serum bottle reactors with working volumes of 100 mL. Before adding the ascorbate reductant, each batch reactor was made up to contain 100 mL of solution containing 32 μM Cr(VI) and ~400 μM Na_2HPO_4 (7 mL of 6.06 mM Na_2HPO_4) as a pH buffer. Reactor volumes were degassed and brought to positive pressure with ultra-high purity N_2 gas for 30 minutes to remove all O_2 and simulate an anaerobic groundwater environment without any reaction due to dissolved O_2. Butyl rubber stoppers prevented air from passing into the bottles. Work by Xu et al., (2004) found minimal change in kinetics of Cr(VI) reduction by ascorbate under varying light sources and ionic strengths.

To begin the reduction reaction, an injection of 0.50 mL of 2.27 mM ascorbate was added to each reactor, which was immediately shaken to ensure complete mixing of reactants prior to significant reaction. Each addition of ascorbate was expected to reduce approximately 4 μM Cr(VI). Later injections resulted in slightly higher initial ascorbate concentrations because the experimental solution decreased in volume as samples were removed (Table 1). Therefore, exact post-injection ascorbate concentrations ranged from 11.6 to 15.1 μM in Expt A and 11.6 to 15.2 μM in Expt B. Reactors were placed on a shaker table at 125 rpm after sampling. The stoichiometry of the Cr(VI)-ascorbate reaction constrained this reaction to consume all ascorbate, while reducing only a fraction of the original Cr(VI). When the reaction of each addition of

ascorbate reached completion and all ascorbate had been oxidized, Cr(VI) samples could be removed and stored without concern about further redox reaction after sampling. This was from 8 to 16 hours between samples. Separate samples were removed to determine pH, Cr(VI) concentration, and $\delta^{53}Cr$.

The process from one ascorbate injection to sampling constituted one "time-step". Time steps were repeated until the remaining Cr(VI) concentration decreased to a level close to the detection limit of the spectrophotometer. Based on the expected reaction stoichiometry, the experiment was designed to be completed with 7 time-steps, yielding 8 total samples (each reactor was sampled once before the first injection to confirm starting Cr(VI) concentration and isotopic composition). Measured concentrations confirmed a consistent relationship between expected and observed Cr(VI) reduction. This time-step method was employed to avoid problems with instability of samples related to the rapid reaction rate of Cr(VI) reduction by ascorbate found in the preliminary experiment (Kitchen et al., 2012).

Sampling syringes and needles were flushed with ultra-high purity N_2 and filled to a volume larger than the sample volume to preserve anoxia and positive pressure in the batch reactors. However, the ascorbate stock was not anoxic, and though ascorbate solutions are known to consume any dissolved oxygen, direct contact with air introduced a small amount of O_2 into the experiment. This is unlikely to have any effect on the experiments, because reactions between O_2 and the Cr species are minimal.

For each time step measurement, 4.2 mL of solution was removed to determine Cr (VI) concentration and 2 to 9 mL was removed for isotopic analysis. Upon removal, samples were passed through a 0.2 μm filter to remove precipitated Cr(III). Cr(VI) concentration was determined immediately, while the isotope subsample was stored up to seven days prior to

purification for mass spectrometry. Because complete consumption of ascorbate was assured, no further steps were needed to prevent additional reduction during storage in the samples taken for isotope analysis.

Uranium(VI) reduction by sulfide

Five anaerobic batch reactor experiments were carried out using methods similar to the reduction experiments performed by Hua et al. (2006). Two lower concentration U(VI) experiments were completed by reacting 140 μM U(VI) with 15 μM NaS (Experiments 1 and 3). Three higher concentration U(VI) experiments were conducted by reacting 280 μM U(VI) with 30 μM NaS (Experiments 2, 4, and 5). Experiments were performed in 130 mL glass serum bottle reactors with working volumes of 100 mL. Before reduction, the five batch reactors each contained a 100 mL solution composed of 140 or 280 μM U(VI), 20 μM PIPES buffer, and 4 mM HCO_3^-. As in the chromium-ascorbate experiments, reactor volumes were degassed by bubbling for 30 minutes with ultra-high purity N_2 gas, and brought to positive pressure.

Based on the expected 4 U(VI): 1 Na_2S stoichiometry (Hua et al., 2006), injections of Na_2S resulting in initial reactor concentrations of 15 μM or 30 μM Na_2S were added to the 140 or 280 μM U(VI) reactors, respectively. This conservative estimate was selected to ensure the reaction allowed for multiple injection, should the stoichiometry not be as predicted. Multiple injections was favorable to control the rate of reaction. Sulfide concentration measurements were used to monitor reaction progress and to ensure U(VI) concentration decrease was due to U(VI) reduction/sulfide oxidation, and not adsorption of U(VI) to solids or precipitation of U(VI) solids. The amount of injected Na_2S represented approximately 30% of the total reductant necessary to fully reduce the U(VI), forming uraninite precipitate and sulfate. These values were calculated to reduce U(VI) in 4 time-steps (3 injections of 15 μM or 30 μM Na_2S and 1 injection

of 7.5 μM or 15 μM Na_2S). The higher concentration reaction (280 μM U(VI) with injections of 30 μM Na_2S) took about 24 hrs to reach completion. To monitor the reaction rate, the first three samples taken in each experiment were measured (from 0 to 3.2 days) after the first injection of Na_2S (15 μM or 30 μM). Once it was clear that sulfide concentration measurements had fallen below the method's detection limit, a second and final injection was added. These "d" samples were sampled four hours after injection due to time constraints. The second injection, corrected for dilution, accounted for the high sulfide concentration of the last data point of each experiment (Table 3). Separate samples were removed to measure pH, sulfide concentration, U(VI) concentration, and $\delta^{238}U$.

As with the chromium-ascorbate experiment, sampling syringes and needles were flushed with ultra-high purity N_2 and filled to a volume larger than the sample volume to preserve anoxia and positive pressure in the anoxic Na_2S stock and batch reactors. Upon removal, samples were passed through a 0.2 μm filter to remove precipitated U(IV) solid (UO_2). Sulfide concentration was determined immediately using the colorimetric method, whereas the U(VI) concentration and isotope subsamples were stored up to five days prior to sample purification for mass spectrometry. Compared to the chromium-ascorbate experiments, the reaction rate of the uranium-sulfide experiments was slow (half-life of ~17 hrs). As a result, some sulfide remained in some samples. To destroy this sulfide, after filtration of the U(IV) precipitate, samples were aerated to oxidize any unreacted sulfide and stop U(VI) reduction.

CONCENTRATION MEASUREMENT

Cr(VI)

Preliminary Cr(VI) concentrations were obtained colorimetrically using US EPA method 7196A. These concentrations were used to determine, for each sample, the proper amount of ^{54}Cr- and ^{50}Cr-bearing "double spike" solution needed for isotope ratio measurements (see below). Cr(VI) and diphenylcarbazide reagent (DPC) form a strong complex with a visible pink color. Visible light absorption at 540 nm measures the concentration of this complex. Samples were acidified to increase the rate of complex formation, and absorbance was measured using a Thermo Genesys spectrophotometer. Absorbance is linear so a single standard and blank can be used for the range of Cr(VI) concentrations. The detection range is approximately 48 to 2 μM, with reproducibility of ±4 μM.

More precise Cr(VI) concentrations, reported in Table 2, were obtained by isotope dilution calculations from the isotope ratio measurements of the samples. These calculations (e.g., Faure and Mensing, 2005) relate the sample's concentration to the measured volume of the sample aliquot prepared for isotopic analysis, the volume of double spike solution added (see below), and precise mass bias-corrected ^{54}Cr/^{52}Cr measurements by the multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS). The ^{54}Cr concentration of the double spike solution was calibrated against a known concentration standard. Uncertainty is approximately ±3%, based on typical reproducibility of this method in this laboratory.

U(VI)

Preliminary U(VI) concentrations used to determine proper double spike addition were measured using a Nu Plasma HR MC-ICP-MS by comparing beam intensity for ^{238}U measured on a Faraday collector for each sample relative to a CRM 112-A standard of known

concentration. As with the Cr experiments, the final reported U(VI) concentrations were obtained from isotope dilution calculations from isotopic measurements. This precision, determined by duplicate measurements on three samples, was ±2.5%.

Sulfide

Sulfide concentrations were measured colorimetrically using US EPA method 376.2. Sulfide reacts with N,N-dimethyl-p-phenylenediamine oxalate in the presence of ferric chloride to produce methylene blue. Visible light absorption at 625 nm measures the concentration of this product. Absorbance is linear up to 31.25 μM so a single standard and blank can be used for the range of sulfide concentrations. Absorbance was measured using a Thermo Genesys spectrophotometer. The detection limit was approximately 3.2 μM with reproducibility of ±1.0 μM.

SAMPLE PREPARATION FOR ISOTOPIC ANALYSIS

Both Cr and U isotope ratio analyses were carried out using a double isotope tracer, or "double spike." Each double spike solution contains two artificially obtained isotopes with a well-calibrated ratio. Chromium has four stable isotopes: ^{50}Cr, ^{52}Cr, ^{53}Cr, and ^{54}Cr. Isotopic ratio studies focus on ^{52}Cr and ^{53}Cr due to their high abundance in nature (83.789% and 9.501% respectively). Our Cr double spike contains ^{50}Cr and ^{54}Cr. With uranium, isotopic ratio studies focus on ^{235}U and ^{238}U due to their high abundance in nature (0.7204% and 99.2742% respectively). The uranium double spike contains two artificially produced isotopes with long half-lives: ^{236}U and ^{233}U.

Addition of the double isotope spike solution to each sample serves two purposes. The measured abundance ratio of these added double spike isotopes is used to correct for any isotope

fractionation during sample preparation and to correct for instrumental mass bias (Ellis et al., 2002; Johnson and Bullent, 2004; Schoenberg et al., 2008). Also, as described above, the measured ratio of the abundance of one of the spike isotopes relative to that of a naturally abundant isotope is used within the isotope dilution method for calculating the precise reported sample concentration.

Sample preparation for Cr isotope measurements followed procedures previously described by Basu et al. (2012). An aliquot of $^{54}Cr/^{52}Cr$ double spike solution was added to each sample prior to purification of Cr and allowed to equilibrate overnight. Cr samples were acidified and passed through a 2 mL bed of BioRad AG1-X8 anion exchange resin, removing the Cr(VI) from solution via adsorption to the resin. Sample matrix was eluted from the columns by passing, sequentially, 1 mL 0.2 M HCl, 15 mL 0.2 M HCl, and 4 mL 2.0 M HCl. Next, Cr(VI) was reduced to Cr(III) by adding 1 mL of 2 M HNO_3 and 3 drops 30% H_2O_2. The Cr(III) effluent, eluted with 6 mL 2 M HNO_3 and 3 drops 30% H_2O_2, was collected after 30 minutes. Samples were dried down, dissolved in concentrated HNO_3, dried down again, then dissolved in 2% HNO_3 for isotope ratio measurement by the MC-ICP-MS.

Sample preparation for U isotope measurements followed procedures previously described by Weyer et al. (2008) and Horwitz (1992). An aliquot of $^{236}U/^{233}U$ double spike solution was added to each sample prior to U purification and allowed to equilibrate overnight. Samples were purified using UTEVA cation-exchange resin (Eichrom). Columns were cleaned with 5 mL 0.05 M HCl and conditioned with 1 mL 3 M HNO_3. Samples were passed through the 0.2 mL bed of resin, removing U(VI) from the solution to the resin. Sample matrix was flushed by a series of 5 mL 3 M HNO_3, 0.6 mL 10 M HCl, and 1 mL 6 M HCl. U was eluted from the resin with 2.8 mL of weak acid (0.05 M HCl) and dried down. The dried sample was dissolved in

concentrated HNO_3 and dried down one last time before dissolution in 2% HNO_3 for isotope ratio measurement by the MC-ICP-MS.

ISOTOPE ANALYSIS

In both Cr and U isotope measurements, double spike data reduction routines were used to correct for instrumental mass bias (Ellis et al., 2002; Johnson and Bullen, 2004; Schoenberg et al., 2008). The double spike method is advantageous over the standard-sample-standard method in providing simultaneous mass bias correction as the sample's isotope ratio is being measured. The ratio of the two spike isotopes ($^{236}U/^{233}U$ or $^{54}Cr/^{50}Cr$) is highly sensitive to the mass bias. In the case of Cr, natural ^{54}Cr and ^{50}Cr also influence the ratio, but the sample and spike can be mathematically separated from each other after the measurements are made. With both Cr and U, small amounts of the isotopes to be determined in the sample (i.e., ^{53}Cr, ^{52}Cr, ^{238}U, and ^{235}U) are present in the spikes, and the final sample compositions are determined after mathematical separation of the spike isotopes from the sample.

Cr isotope measurements were performed using methods developed by Schoenberg et al. (2008) and described in more detail in Kitchen et al. (2012) and Basu et al. (2012). Mass spectrometry was carried out using a Nu Plasma HR MC-ICP-MS in pseudo-high resolution mode. Samples were introduced as 2% HNO_3 solutions into the plasma using a DSN-100 desolvating nebulizer. Interferences cause by Fe, V, and Ti isotopes were measured and corrected for in each analysis. A purified standard solution (NIST Cr standard SRM 979) of known isotopic composition was measured after every four samples, and measurements were normalized to the daily mean value of SRM 979. Typical precision attained using these methods in the same laboratory was roughly ±0.1‰.

U isotope measurements were performed using the Nu Plasma MC-ICP-MS in low-resolution mode (Shiel et al., 2013)**.** Sample introduction was the same as Cr. U isotope standard CRM 112-A with known isotope composition was measured after every three samples. Sample measurements were normalized to CRM 112-A, thus correcting for small instrumental drift. The uncertainty of the isotope measurements was ±0.15‰ based on twice root mean square difference for three pairs of duplicates and 6 samples of known $\delta^{238}U$ (t=0 samples with $\delta^{238}U$ = 0.00‰).

RAYLEIGH DISTILLATION MODEL

The Rayleigh distillation model relates the shift of the isotope ratio to the extent of reduction, assuming a well-mixed reaction vessel without interaction between the reaction product and the reactant. Accordingly, the $\delta^{53}Cr$ (or $\delta^{238}U$) values and concentration data were fit to the Rayleigh distillation relationship:

$$\delta(t) = (\delta_0 + 1000)\left(\frac{C(t)}{C_0}\right)^{(\alpha-1)} - 1000 \qquad \textbf{(5)}$$

where δ(t) and C(t) are the isotopic delta value and concentration at some time t after the reaction began, δ_0 and C_0 are the initial isotopic delta value and concentration, and α is the isotopic fractionation factor. α is calculated from experimental data using the slope of the best-fit line to the [ln($\delta^{53}Cr$)+1000] vs. ln(C(t)) plot, using the method given in Scott et al. (2004).

RESULTS

CR(VI) REDUCTION BY ASCORBATE

Results from the Cr(VI) reduction experiments are given in Tables 1 and 2. The reaction rate observed in the preliminary experiment was rapid (Fig. 1); this is consistent with results reported by Xu et al. (2004). In the preliminary experiment, approximately 80% of starting Cr(VI) was reduced within the first 5 minutes of the reaction. According to the concentration measurements, nearly all Cr(VI) was removed from the system within 40 minutes. This reaction rate is too fast to allow isotopic analysis of time series samples taken after a single, large addition of reductant. A sample taken at a certain moment in time would be subject to additional reduction and isotopic fractionation after measurement of concentration but before sample preparation. Accordingly, the stepwise ascorbate injection regimen described above was essential to the success of the experiments.

As expected, the stepwise method of ascorbate addition controlled reaction progress, as each step was allowed to proceed to completion and samples taken after complete consumption of reductant were stable. Also, because less ascorbate was added for each injection, as compared to the preliminary experiment, the Cr(VI) reduction rates were slower, as lower concentration results in slower rate. The Cr(VI) concentration decreases induced by the repeated additions were somewhat less than those predicted by the reaction stoichiometry reported by Xu et al. (2004). Table 2 lists samples taken, their concentrations, and measured δ^{53}Cr values.

The plot of δ^{53}Cr versus Cr(VI) concentration (Fig. 2) follows a nearly linear trend with slight upward curvature. A plot of ln(δ^{53}Cr+1000‰) vs. ln[Cr(VI)] concentration), Fig. 3, shows a strong linear trend between t=0 and t=4. Thus, the data are consistent with a Rayleigh distillation model up to about 60% reduction. Later data exhibit some scatter and all fall below

the trend set by the first 4 data points in each experiments (Fig. 3). Inconsistency of these data relative to the earlier samples is attributed to passage of some Cr(III) through the filters or incomplete reduction (see discussion). Therefore, these points were ignored when the data were fit to calculate the isotopic fractionation (ε value). The resulting ε values are -2.83‰ ±0.05‰ for experiment A and -3.16‰ ±0.23‰ for experiment B; uncertainties were calculated from scatter of the data about the best-fit line, using standard linear estimation methods.

U(VI) REDUCTION BY SULFIDE

Results from the U(VI) reduction experiments are given in Tables 3 and 4, and in Figs. 4, 5, and 6. With one batch reactor at the higher U(VI) concentration (experiment 2; 280 μM U(VI) with 30 μM NaS), sulfide concentration was monitored after the first sulfide addition to determine reaction rate (Table 3; Fig. 4). The gradual disappearance of sulfide over >24 hours demonstrates that the reaction rate was not so rapid that reaction might be diffusion-limited during initial mixing of the sulfide solution into the U(VI) solution (see discussion).

Initial delta values are, as expected, within error of 0.00‰. The mean $\delta^{238}U$ of dissolved U(VI) in the samples taken immediately before the second sulfide injection was 0.03‰ with standard deviation of ±0.09‰ and standard error of 0.04‰. Mean delta value of the last data points collected from all experiments (t = 2.9 to 3.7 days; 40% to 65% reduction of U(VI)) was 0.10‰ with standard deviation of ±0.07‰ and standard error of ±0.03‰. Although the mean of the reacted samples is slightly higher than the mean of the initial samples, the uncertainties overlap. Thus, a consistent increase in $\delta^{238}U$ is not statistically supported by the data, despite up to 65% reduction of the initial U(VI). Results are plotted for the lower concentration experiments in Fig. 5; those from the higher concentration experiments are given in Fig. 6.

DISCUSSION

APPLICABILITY TO NATURAL WATERS

The experiments performed were designed to be very simple buffered solutions roughly consistent with groundwater pH values. Therefore, their applicability to natural settings is somewhat limited by experimental parameters. For example, the Cr experiment, compared to a groundwater aquifer, has a very high concentration of ascorbate and lacks a complex matrix of other ions. However, Cr(VI) reduction rates by ascorbate (Xu et al., 2004) and sulfide (Kim et al., 2001) are not significantly altered with varying ionic strengths of 0.01-1M. Accordingly, it seems unlikely that the magnitude of isotopic fractionation would be sensitive to ionic strength. We suggest that the results obtained in this study apply to the range of ionic strengths observed in groundwater systems. Further experiments could test this hypothesis.

In the U experiments, the concentration of U(VI) used was much higher than applicable environmental conditions. However, the necessity to measure isotopes in a timely manner required a certain minimum U(VI) concentration. Future experiments can be improved by altering these experimental parameters to match realistic groundwater conditions.

POSSIBLE DEFECTS IN THE CR(VI)-ASCORBATE EXPERIMENTS

Samples were stored up to 7 days prior to preparation for isotopic analysis. This storage time allowed Cr(VI) and Cr(III) the possibility to exchange isotopes, altering the measured isotopic ratios. However, the rate of Cr(VI)-Cr(III) equilibrium isotope effect has been shown to be orders of magnitude less than the minimum rate that could impact these experiments (Wang, 2013; Zink et al., 2010). For example, isotopic equilibrium of an experiment with Cr(VI) concentration of 0.2 M, pH of 1.2, and 40°C was achieved in roughly 500 days (Wang, 2013).

The rate of equilibration of these stored samples at lower temperatures and concentrations would be much slower.

Light was not excluded from these experiments. Consequently, there is some possibility that photochemical reactions occurred in addition to the targeted non-photochemical reduction of Cr(VI) by ascorbate. However, the laboratory light generated by standard fluorescent fixtures should have had minimal effects on these experiments. Though irradiation sometimes catalyzes chemical reactions, Xu et al., (2004) found the rate of Cr(VI) reduction by ascorbate under different light sources to be nearly indistinguishable from those performed in the absence of light. Therefore we conclude that effects of photochemical reactions were probably negligible despite some exposure to light in the ca. 60 minutes required for Cr(VI) reduction to go to completion.

The final data points in each experiment deviate from the linear trend set by the earlier data points in Fig. 3. The early data points follow the expected Rayleigh distillation model within analytical uncertainty, but all later data points are shifted to lower δ^{53}Cr relative to the Rayleigh model, to varying extents. One possible cause of this deviation is rapid approach toward isotopic equilibrium between Cr(VI) and Cr(III), but as discussed above, exchange rates are very slow and thus we dismiss isotopic exchange as a potential cause. A more likely cause is Cr(III) nanoparticles or Cr(III)-ascorbate complexes that could have passed through the 0.2 μm filter and then moved through the ion exchange process with the Cr(VI). Possibly, these Cr(III) nanoparticles or complexes oxidized to Cr(VI) during storage of samples. If a small fraction of the Cr(III) was included with Cr(VI) that was analyzed for isotope ratios, early samples would not be much affected because of their high Cr(VI) to Cr(III) ratios, while later samples would be much more susceptible due to their low Cr(VI) to Cr(III) ratios. This would lead to the lower

than expected Cr(VI) δ^{53}Cr values toward the end of the experiments. This fits the observed pattern.

Although we cannot conclusively identify the processes occurring toward the end of the experiments, the early data points fit the expected Rayleigh trend and we strongly suggest that they precisely indicate the isotopic fraction. The apparently random scatter of the later data points suggests a sample preparation artifact caused errors in their isotopic compositions. Given the suspected problems with separation of Cr(VI) and Cr(III), it seems reasonable to exclude the later data points. It is possible that the latest data points not excluded from the regressions are slightly affected by the same problem(s), but because these points conform well to the linear trend of the earlier points, the errors must be very small and the effect on the calculated isotopic fractionation must also be very small.

POSSIBLE DEFECTS IN THE URANIUM-SULFIDE EXPERIMENTS

In these experiments, Na_2S was added to the experiment between 1 and 21 hours after the other reagents (U(VI) stock, PIPES buffer, bicarbonate) were mixed together. In those experiments with less equilibration time prior to the onset of reduction, it is possible the uranium and bicarbonate may not have had time for the distribution of strong uranium-carbonate complexes to fully equilibrate before U(VI) reduction began. However, the U(VI) stock solution used for the experiments contained 100 mM bicarbonate, forming U(VI)-carbonate complexes well before experiments began. Thus, while the distribution of the various U(VI)-carbonate complexes may have changed in the early stages of the experiments, the U(VI) was always well complexed with carbonate ions. Adsorption of U(VI) onto solid surfaces should have been negligible. Furthermore, the experiments lasted several days, during which time the U(VI)-

carbonate complexes would have fully equilibrated. As none of the isotopic results show significant change during the course of the experiments, there is no evidence for significant isotopic effects resulting from U(VI) speciation changes in the early stages.

Some of the samples were taken prior to complete reaction of the sulfide. Accordingly, efforts were made to thoroughly oxidize all sulfide prior to sample storage, in order to stop U(VI) reduction. However, an unknown amount of sulfide remained in some samples, evidenced by a slight sulfide smell in eluted solutions after sample purification. Residual unreacted sulfide in the samples would cause additional loss of U(VI) after sampling. However, because the concentration measurements were determined by isotope dilution with the double spike, reduction occurring after sampling but before spiking (a time period of up 4 days) is still recorded. Reduction occurring after spiking would have been corrected by the double spike procedure, which extracts, from measured $^{236}U/^{233}U$ ratios, both the instrumental mass bias and any isotopic fractionation induced by sample preparation. It is thus expected that small amounts of post-spiking reduction have little effect on the final result. More importantly, we observed no significant isotopic ratio shift in these experiments, so clearly, any effects of extra reduction beyond the amount that occurred prior to spiking are not a concern.

Finally, U(IV) particles removed via filtration could have provided sorption sites for U(VI), thereby removing some U(VI) from solution without reduction and driving the remaining dissolved U(VI) to an isotopically heavier δ value. However, the UO_2 generated was very small, so sorption would not have been able to remove much U(VI). The findings of Brennecka et al., (2011b) indicate an isotopic fractionation in $\delta^{238}U$ of ~0.2‰ for adsorption of U(VI) onto K-birnessite, where adsorbed U is isotopically lighter than dissolved.

STOICHIOMETRY OF CHROMIUM-ASCORBATE EXPERIMENTS

Cr(VI) losses from solution were compared to the losses expected via reaction with the known amounts of ascorbate injected, to determine if the measured decrease in Cr(VI) concentration was due to reduction or adsorption. Xu et al. (2004) supports a stoichiometry consistent with the following reaction:

$$2Cr(VI) + 3\text{ascorbic acid} + 8\ H^{+} \rightarrow 2\ Cr^{3+} + 3\text{dehydroscorbic acid} + 7H_2O$$

Each addition of ascorbate to the stepwise experiments was 0.50 mL of a 2.27 mM stock. As ascorbate addition and sampling added and removed solution from the originally 100.0 mL batch reactors, we performed dilution calculations to determine the extent and stoichiometry of the reaction. In all cases, the amount of Cr(VI) lost from solution was less than that predicted from the reaction stoichiometry. Furthermore, this discrepancy increased over time in the experiments, with the last additions driving the ascorbate concentrations above 15 micromolar but only causing loss of 3 micromolar Cr(VI). This is probably caused by a loss of potency of the ascorbate stock, which contained dissolved oxygen when it was made and was exposed to air during the experiments. Thus it is possible that the ascorbate stock was of lower concentration than calculated based on the measured mass of reagent weighed. Because the true strength strength of the ascorbate acid is unknown, the reaction stoichiometry does not provide a means to rule out some Cr(VI) loss due to sorption or coprecipitation of Cr(VI) to the expected Cr(III) precipitate. However, Cr(VI) adsorption is expected to be negligible in the presence of phosphate ions from the Na_2HPO_4 buffer. Thus, we are confident that all Cr(VI) loss is due to reduction.

STOICHIOMETRY OF URANIUM-SULFIDE EXPERIMENTS

In all experiments, colorimetric concentration measurements of sulfide show a decrease in sulfide that coincides with decrease in U(VI) concentration determined by the isotope dilution method. This decrease supports the interpretation that the drop in U(VI) concentration is due to reduction by sulfde and not other processes. Experiment 2 was treated identically to the other higher concentration experiments (4 and 5) with the exception of additional early sampling to determine sulfide concentrations. The actual sulfide concentration based on spectrophotometer measurement at t=0 was 26.2 μM. Figure 4 shows the decrease in sulfide concentration over the first 51.3 hours of experiment 2. The colorimetrically determined sulfide concentration measurement decreased to about 8 micromolar over 28 hours and then did not appear to change for the next 25 hours. This trend suggests a measurement problem, as U(VI) was present throughout this time and continued to decrease after 53 hours. Therefore, it seems highly likely that the 28 hour measurement was inaccurate. This measurement was disregarded and reaction stoichiometry was determined for the 51.3 hour sample. Actual uranium concentrations, based on the isotope dilution method, were 334.2 and 214.2 μM for t=0 and t=51.3 hours, respectively. Taking into consideration the loss of 120 μM U from solution and the 18 μM loss of sulfide, the calculated stoichiometric ratio is 6.7:1.

Hua et al. (2006) list three possible reaction mechanisms for reduction of U(VI) by HS^- with distinct stoichiometries:

$$12UO_2(CO_3)_3^{4-} + HS^- + 47H^+ \rightarrow 4U_3O_{8(s)}\ \text{(pitchblende)} + SO_4^{2-} + 36CO_{2(g)} + 24H_2O \quad (1)$$

$$4UO_2(CO_3)_3^{4-} + HS^- + 15H^+ \rightarrow 4UO_{2(s)}\ \text{(uraninite)} + SO_4^{2-} + 12CO_{2(g)} + 8H_2O \quad (2)$$

$$UO2^{2+} + HS^- \rightarrow UO_2 + S^0\ \text{(elemental sulfur)} + H^+ \quad (3)$$

Hua et al. (2006) found that the equation (2) is the one best represented by the results. In the experiments of the present study, under conditions similar to those of Hua et al. (2006), the calculated molar ratio of S loss to U loss of 6.7:1 is somewhat greater than the 4:1 ratio demanded by the second reaction. This may be due to analytical errors, which, as described above, seem to have occurred in at least one sulfide measurement. If the analyses are correct, though, the result suggest that some of the U lost from solution was U(VI). This could have occurred if some U_3O_8 was precipitated as in the reaction above, or if U(VI) was lost to adsorption in the experiments. Accordingly, the current data set does not allow precise quantification of U(VI) reduction from the decrease in sulfide concentration. However, the measured loss of 18 μM sulfide in experiment 2 indicates that at least 72 μM U(VI) should have been reduced over 51.3 hours. Additional reduction occurred after this time, so most likely at least 30% of the U(VI) was reduced by the end of the experiment.

MECHANISMS OF URANIUM ISOTOPE FRACTIONATION

Uranium isotope fractionation is induced in redox transformations (Stirling et al., 2007; Weyer et al., 2008; Bopp et al., 2009; Basu et al., 2014). Uranium isotopes fractionate based on differences in nuclear volume and mass. Mass dependent, kinetic fractionation results from the tendency of lighter isotope to react at greater rates**.** Kinetic fractionation of uranium isotopes from the nuclear volume effect results from the tendency of isotopes with larger nuclei (which correspond to the heavier isotopes) to preferentially react (Bopp et al., 2009; Schauble, 2007). Accordingly, the two isotopic fractionation mechanisms operating during U(VI) reduction oppose each other. Theory-based calculations for isotopic equilibrium between U(VI) and U(IV) indicate the nuclear volume effect is stronger than the mass-dependent effect, resulting in

isotopically heavy U(VI). But isotopic equilibrium fractionation does not necessarily indicate the direction of kinetic isotope fractionation, which has been determined in several laboratory and field experiments. Studies published to date indicate that reduction produces isotopically heavy U(IV) in a few different settings (Basu et al., 2014; Bopp et al., 2010; Stirling, 2007). Thus it appears that, in at least some U(VI) reduction reactions, the nuclear volume effect dominates over mass dependent effects during kinetic U isotope fractionation.

Because the two mechanisms of U isotope fractionation oppose each other, the absence of change in measured $\delta^{238}U$ in the present study could result from the combination of the two mechanisms. Although microbial reduction of U isotopes during reduction of U(VI) has been shown to induce a kinetic fractionation with ^{238}U reacting at a greater rate than ^{235}U (Basu et al., 2014), abiotic reduction by sulfide could have a very different mechanism of reduction. Currently, specific information about the reaction mechanisms of both types of reduction is poorly understood, and it is impossible to explain confidently the cause of the difference in isotopic fractionation. However, a basic understanding of the systematics of isotopic fractionation exists, and some speculation about possible reasons for the lack of fractionation observed in these experiments seems warranted. The kinetic isotopic fractionation of a chemical reaction consisting of multiple steps has been explored theoretically and experimentally. The magnitude of isotopic fractionation of the overall reaction is determined by adding the isotopic effects of all reaction steps up to and including the rate-limiting step (Rees, 1973; Canfield, 2001). Therefore, the observed overall isotopic fractionation can be small if the rate-limiting step is early in the chain of steps and does not fractionate isotopes. This is plausible since uranium has complicated configurations of oxygen and electrons, and multiple steps of coordination changes and electron transfers are certainly involved in reduction of U(VI) to U(IV). However,

we do observe systematic fractionation during microbial reduction. The situation with uranium is particularly complex relative to elements like Cr, because mass dependent fractionation is expected to respond to changes in vibrational energies of bonds, whereas nuclear volume effects are driven by electron orbital changes. Therefore, the fact that isotopic fractionation during sulfide-driven reduction is very different from that during microbial reduction is perhaps not surprising. Further study of the reaction mechanisms and those of other reactions should help shed light on this phenomenon.

INTERPRETATION OF URANIUM ISOTOPE DATA

With the exception of samples 2-d and 4-d, all $\delta^{238}U$ values within each experiment are indistinguishable from initial $\delta^{238}U$ of 0.00‰ based on a 95% confidence interval of ± 0.15‰. However, in Fig. 7, there appears to be a weak trend in the data, with the remaining U(VI) becoming slightly isotopically heavier as reduction increases, and two of the last three samples significantly heavier than the starting material. Because this trend is so weak and the analytical uncertainty is not perfectly known, it is not possible to be certain if any isotopic fractionation occurred. It is possible to attain better U isotope measurement precision, and future work should be done to explore the quantification of any very slight isotopic fractionation during reduction of U(VI) by sulfide. However, the data of the present study indicate that the relatively large isotopic fractionation observed in microbial reduction and bioreduction of waters experiments is absent in this reaction. In the microbial reduction experiments of Basu et al. (2014), similar extents of reduction produced $\delta^{238}U$ shifts of about 0.7‰ to 1.0‰.

CONCLUSIONS

The results from the Cr(VI) batch reactor experiments demonstrate significant isotopic fractionation consistent with a mass-dependent kinetic isotope effect during Cr(VI) reduction by ascorbate. According to the two experiments performed, the magnitudes of isotopic fractionation (ε) were found to be -2.85‰ and -3.16‰. These results are within the range of isotopic fractionation previously reported for Cr(VI) reduction by other abiotic materials, ranging from -2.11‰ for FeS to -3.91‰ for goethite (Basu and Johnson, 2012; Kitchen et al., 2012; Berna et al., 2010; Ellis et al., 2002). They are also well within the range of isotopic fractionation for organic reductants found by Kitchen et al. (2012).

The results from experiments in which U(VI) was reduced by dissolved sulfide show very little shift in $\delta^{238}U$ with up to 60% reduction. The absence of strong isotopic fractionation during reduction by sulfide, an abiotic reductant, contrasts with significant change in $\delta^{238}U$ during reduction by microbes (Basu et al., 2014). This is supported by recent work by Stylo et al. (2014) which finds no isotopic fractionation for reduction of U(VI) by FeS. This could imply an exciting distinction between biotic and abiotic reduction of U(VI). If microbial reduction always fractionates U isotope ratios but abiotic reduction does not, then isotopic signatures could be used to identify microbial reduction is occurring. On the other hand, if no fractionation accompanies a decrease in U(VI) concentration, this does not confirm abiotic reduction, as dilution or adsorption could cause the same pattern.

A difference in isotopic fractionation would also suggest a major difference in the mechanism of U(VI) reduction, i.e., that microbes reduce U(VI) in a different manner than does abiotic sulfide reduction. Future experiments reducing U(VI) by other abiotic materials (FeS,

Fe^{2+}, magnetite, etc.) are critical in exploring the hypothesis that all abiotic U(VI) reduction reactions induce little isotopic fractionation.

REFERENCES

Abe M., Suzuku T., Fujii Y., Hada M., Hirao K. (2008) An ab initio molecular orbital study of the nuclear volume effects in uranium isotope fractionations. Journal of Chemical Physics. **129(16),** 164309.

Asatiani N. V., Abuladze M. K., Kartvelishvili T. M., Bakradze N. G., Sapojnikova N. A., Tsibakhashvili N. Ya., Tabatadze L. V., Lejava L. V., Asanishvili L. L., Holman H. (2004) Effect of Chromium(VI) Actions on Anthrobacter oxydans. Current Microbiology. **49(5),** 321-326.

Basu A. and Johnson T. M. (2012) Determination of hexavalent chromium reduction using Cr stable isotopes: Isotopic fractionation factors for permeable reactive barrier materials. Environ. Sci. Technol. **46,** 5353-5360.

Basu, A., Sanford, R. A., Johnson, T. M., Lundstrom, C. C., Löffler, F. E., (2014) Uranium isotopic fractionation factors during U(VI) reduction by bacterial isolates. Geochimica et Cosmochimica Acta **136**, 100-113.

Berna E. C., Johnson T. M., Makdisi R. S. and Basu A. (2009) Cr stable isotopes as indicators of Cr (VI) reduction in groundwater: A detailed time-series study of a point-source plume. Environ. Sci. Technol. **44,** 1043-1048.

Benes P. (1999) The environmental impacts of uranium mining and milling and the methods of their reduction. Chem. Separation Technol. and Related Methods **53,** 225-246.

Bigeleisen J. (1996) Nuclear size and shape effects in chemical reactions. Isotope chemistry of the heavy elements. J. Am. Chem. Soc. **118,** 3676-3680.

Blowes D. W. (2002) Tracking hexavalent Cr in groundwater. Science, **295**, 2024-2025.

Bopp C. J., Lundstrom C. C., Johnson T. M. and Glessner J. J. G. (2009) Variations in $^{238}U/^{235}U$ in uranium ore deposits: Isotopic signatures of the U reduction process? Geology **37,** 611-614.

Bopp IV C. J., Lundstrom C. C., Johnson T. M., Sanford R. A., Long P. E. and Williams K. H. (2010) Uranium $^{238}U/235U$ isotope ratios as indicators of reduction: results from an in situ biostimulation experiment at Rifle, Colorado, USA. Environ. Sci. Technol. **44,** 5927-5933.

Brennecka G. A., Borg L. E., Hutcheon I. D., Sharp M. A. and Anbar A. D. (2010) Natural variations in uranium isotope ratios of uranium ore concentrates: Understanding the $^{238}U/^{235}U$ fractionation mechanism. Earth Planet. Sci. Lett. **291,** 228-233.

Brennecka G. A., Herrmann A. D., Algeo T. J. and Anbar A. D. (2011a) Rapid expansion of oceanic anoxia immediately before the end-Permian mass extinction. Proceedings of the National Academy of Sciences **108,** 17631-17634.

Brennecka G. A., Wasylenki L. E., Bargar J. R., Weyer S. and Anbar A. D. (2011b) Uranium isotope fractionation during adsorption to Mn-oxyhydroxides. Environ. Sci. Technol. **45,** 1370-1375.

Canfield D. E. (2001) Biogeochemistry of sulfur isotopes; Stable isotope geochemistry. Reviews in Mineralogy and Geochemistry **43,** 607-636.

Clark S. K. and Johnson T. M. (2008) Effective isotopic fractionation factors for solute removal by reactive sediments: a laboratory microcosm and slurry study. Environ. Sci. Technol. **42,** 7850–7855.

De Flora S. (2000) Threshold mechanisms and site specificity in chromium (VI) carcinogenesis. Carcinogenesis **21,** 533-541.

Døssing L. N., Dideriksen K., Stipp S. L. S. and Frei R. (2011) Reduction of hexavalent chromium by ferrous iron: a process of chromium isotope fractionation and its relevance to natural environments. Chem. Geol. **285,** 157–166.

Druhan J. L., Conrad M. E., Williams K. H., N'Guessan L., Long, P. E., Hubbard S. S. (2008) Sulfur Isotopes as indicators of amended bacterial sulfate reduction processes influencing field scale uranium bioremediation. Environ. Sci. Technol. **42(21),** 7842-7849.

Du X., Boonchayaanant B., Wu W., Fendorf S., Bargar J. and Criddle C. S. (2011) Reduction of uranium (VI) by soluble iron (II) conforms with thermodynamic predictions. Environ. Sci. Technol. **45,** 4718-4725.

Ellis A. S., Johnson T. M. and Bullen T. D. (2002) Cr isotopes and the fate of hexavalent chromium in the environment. Science **295,** 2060–2062.

Ellis A. S., Johnson T. M. and Bullen T. D. (2004) Using chromium stable isotope ratios to quantify Cr(VI) reduction: lack of sorption effects. Environ. Sci. Technol. **38,** 3604–3607.

Faure, G. and Mensing, T.M. (2005) Isotopes: Principles and Applications. John Wiley & Sons, Inc.: Hoboken. **1,** pp. 896.

Ferraiolo G., Zilli M. and Converti A. (1990) Fly ash disposal and utilization. Journal of Chemical Technology and Biotechnology **47,** 281-305.

Frei R., Gaucher C., Poulton S. W. and Canfield D. E. (2009) Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes. Nature **461,** 250–253.

Horwitz E. P., Dietz M. L., Chiarizia R. and Diamond H. (1992) Separation and preconcentration of uranium from acidic media by extraction chromatography. Anal. Chim. Acta **266,** 25–37.

Hua B. and Deng B. (2008) Reductive immobilization of uranium (VI) by amorphous iron sulfide. Environ. Sci. Technol. **42,** 8703-8708.

Hua B., Xu H., Terry J., Deng B. (2006) Kinetics of Uranium(VI) Reduction by hydrogen sulfide in anoxic aqueous systems. Environ. Sci. Technol. **40,** 4666-4671.

Ithurbide A., Peulon S., Miserque F., Beaucaire C. and Chaussé A. (2009) Interaction between uranium (VI) and siderite (FeCO3) surfaces in carbonate solutions. Radiochimica Acta **97,** 177-180.

Izbicki J. A., Ball J. W., Bullen T. D. and Sutley S. J. (2008) Chromium, chromium isotopes and selected trace elements, western Mojave Desert, USA. Appl. Geochem. **23,** 1325–1352.

Johnson T. M., and Bullen T. D. (2004) Mass-dependent fractionation of selenium and chromium isotopes in low-temperature environments. In Geochemistry of non-traditional stable isotopes. Johnson, C.M., Beard, B. L., Albarede, F., Eds.; Mineralogical Society of America: Washington, DC, pp. 289–317.

Kim C., Zhou Q., Deng B., Thornton E. C., and Xu H. (2001) Chromium(VI) Reduction by Hydrogen Sulfide in Aqueous Media: Stoichiometry and Kinetics. Environ. Sci. Technol. **35(11),** 2219-2225.

Kitchen J. W., Johnson T. M., Bullen T. D., Zhu J. and Raddatz A. (2012) Chromium Isotope Fractionation Factors for Reduction of Cr (VI) by Aqueous Fe (II) and Organic Molecules. Geochim. Cosmochim. Acta. **89,** 190-201.

Losi M., Amrhein C. and Frankenberger Jr W. (1994) Environmental biochemistry of chromium. Rev. Environ. Contam. Toxicol. **136,** 91.

Lyons T. W., Anbar A. D., Severmann S., Scott C. and Gill B. C. (2009) Tracking euxinia in the ancient ocean: a multiproxy perspective and Proterozoic case study. Ann. Rev. Earth Planet. Sci. **37,** 507-534.

Markich S. J. (2002) Uranium speciation and bioavailability in aquatic systems: an overview. Sci. World J. **2,** 707-729.

Missana T., Maffiotte C. and Garcı!a-Gutiérrez M. (2003) Surface reactions kinetics between nanocrystalline magnetite and uranyl. J. Colloid Interface Sci. **261,** 154-160.

Montoya-Pino C., Weyer S., Anbar A. D., Pross J., Oschmann W., van de Schootbrugge B. and Arz H. W. (2010) Global enhancement of ocean anoxia during Oceanic Anoxic Event 2: A quantitative approach using U isotopes. Geology **38,** 315-318.

Morales, D. K., Ocampo, W., and Zambrano, M. M. (2007) Efficient removal of hexavalent chromium by a tolerant Streptomyces sp. affected by the toxic effect of metal exposure. J. Appl. Microbiol. **103,** 2704-2712.

Murphy, W. M., & Shock, E. L. (1999). In Uranium: Mineralogy, Geochemistry and the Environment. Reviews in Mineralogy, Eds. PC Burns and R. Finch, Mineralogical Society of America, **38**, 221-253.

Nriagu J. O. and Nieboer E. (1988) Chromium in the natural and human environments. Adv. Environ. Sci. Technol. **20,** 571.

O'Loughlin E. J., Kelly S. D., Cook R. E., Csencsits R. and Kemner K. M. (2003) Reduction of uranium (VI) by mixed iron (II)/iron (III) hydroxide (green rust): formation of UO2 nanoparticles. Environ. Sci. Technol. **37,** 721-727.

Oze C., Bird D. K. and Fendorf S. (2007) Genesis of hexavalent chromium from natural sources in soil and groundwater. Proc. Natl. Acad. Sci. USA **104,** 6544–6549.

Pettine, M., D'Ottone, L., Campanella, L., Millero, F.J., Passino, R., (1998). The reduction of chromium (VI) by iron (II) in aqueous solutions. Geochim. Cosmochim. Acta **62(9),** 1509–1519.

Proctor, D. M., Harris, M. A. and Finley, B. L. (eds.) (2000) Chromium in soil: perspectives in chemistry, health, and environmental regulation. J. Soil Contam. **6,** 557–797.

Raddatz A. L., Johnson T. M. and McLing T. L. (2010) Cr stable isotopes in Snake River Plain Aquifer groundwater: Evidence for natural reduction of dissolved Cr(VI). Environ. Sci. Technol. **45,** 502–507.

Rademacher L. K., Lundstrom C. C., Johnson T. M., Sanford R. A., Zhao J. and Zhang Z. (2006) Experimentally determined uranium isotope fractionation during reduction of hexavalent U by bacteria and zero valent iron. Environ. Sci. Technol. **40,** 6943-6948.

Rees C. E. (1973) A steady-state model for sulphur isotope fractionation in bacterial reduction processes. Geochim. Cosmochim. Acta **37,** 1141-1162.

Robles-Camacho, J. and Armienta, M.A. (2000) Natural chromium contamination of groundwater at Leon Valley, Mexico. J. Geochem. Explor. **68,** 167-181.

Schauble E., Rossman G. R. and Taylor H. P. (2004) Theoretical estimates of equilibrium chromium-isotope fractionations. Chem. Geol. **205,** 99–114.

Schauble E. A. (2007) Role of nuclear volume in driving equilibrium stable isotope fractionation of mercury, thallium, and other very heavy elements. Geochim. Cosmochim. Acta **71,** 2170-2189.

Schoenberg R., Zink S., Staubwasser M. and von Blanckenburg F. (2008) The stable Cr isotope inventory of solid Earth reservoirs determined by double spike MC–ICP–MS. Chem. Geol. **249,** 294–306.

Scott K., Lu X., Cavanaugh C. and Liu J. (2004) Optimal methods for estimating kinetic isotope effects from different forms of the Rayleigh distillation equation. Geochim. Cosmochim. Acta **68,** 433-442.

Shiel A. E., Laubach P. G., Johnson T. M., Lundstrom C. C., Long P.E. and Williams K.H. (2013) No measureable changes in $^{238}U/^{235}U$ due to the adsorption of U(VI) from groundwater at the Rifle, Colorado IFRC Site. Environ. Sci. Technol. **47(6),** 2535-2541.

Sikora E. R., Johnson T. M., and Bullen T. D. (2008) Microbial mass-dependent fractionation of chromium isotopes. Geochim. Cosmochim. Acta **72,** 3631–3641.

Stearns D. M., and Wetterhahn, K. E. (1994) Reaction of Cr(VI) with ascorbate produces chromium(V), chromium(IV), and carbon-based radicals. Chem. Res. Toxicol. **7**, 219–230.

Stirling C. H., Andersen M. B., Potter E. K., and Halliday A. N. (2007) Low-temperature isotopic fractionation of uranium. Earth Planet. Sci. Lett. **264,** 208-225.

Stylo M., Neubert N., Wang Y., Weyer S., and Bernier-Latmani R. (2014) Uranium Isotope Fractionation and Signature for Biotic Reduction Processes. 24th annual V. M. Goldschmidt Conference, Sacramento, USA.

U.S. Environmental Protection Agency (1998) Toxicological Review of Trivalent Chromium. National Center for Environmental Assessment, Office of Research and Development, Washington, DC.

Viamajala S., Peyton B. M., Apel W. A., and Petersen J. N. (2002) Chromate/nitrite interactions in Shewanella oneidensis MR- 1: Evidence for multiple hexavalent chromium [Cr (VI)] reduction mechanisms dependent on physiological growth conditions. Biotechnol. Bioeng. **78,** 770-778.

Wang, X., and Johnson, T.M. (2011), Interpretation of chromium isotopic data: Exchange kinetics and fractionation factors between Cr(III) and Cr(VI). Abstract H21A-1048 presented at 2011 Fall Meeting, AGU, San Francisco, Calif., 5-9 Dec.

Weyer S., Anbar A., Gerdes A., Gordon G., Algeo T. and Boyle E. (2008) Natural fractionation of $^{238}U/^{235}U$. Geochim. Cosmochim. Acta **72,** 345-359.

Xu X., Li H., Li X., and Gu J. (2004) Reduction of hexavalent chromium by ascorbic acid in aqueous solutions. Chemosphere **57(7),** 609-613.

Zink S., Schoenberg R., Staubwasser M., 2010. Isotopic fractionation and reaction kinetics between Cr(III) and Cr(VI) in aqueous media. Geochim. Cosmochim. Acta **74,** 5729-5745.

FIGURES AND TABLES

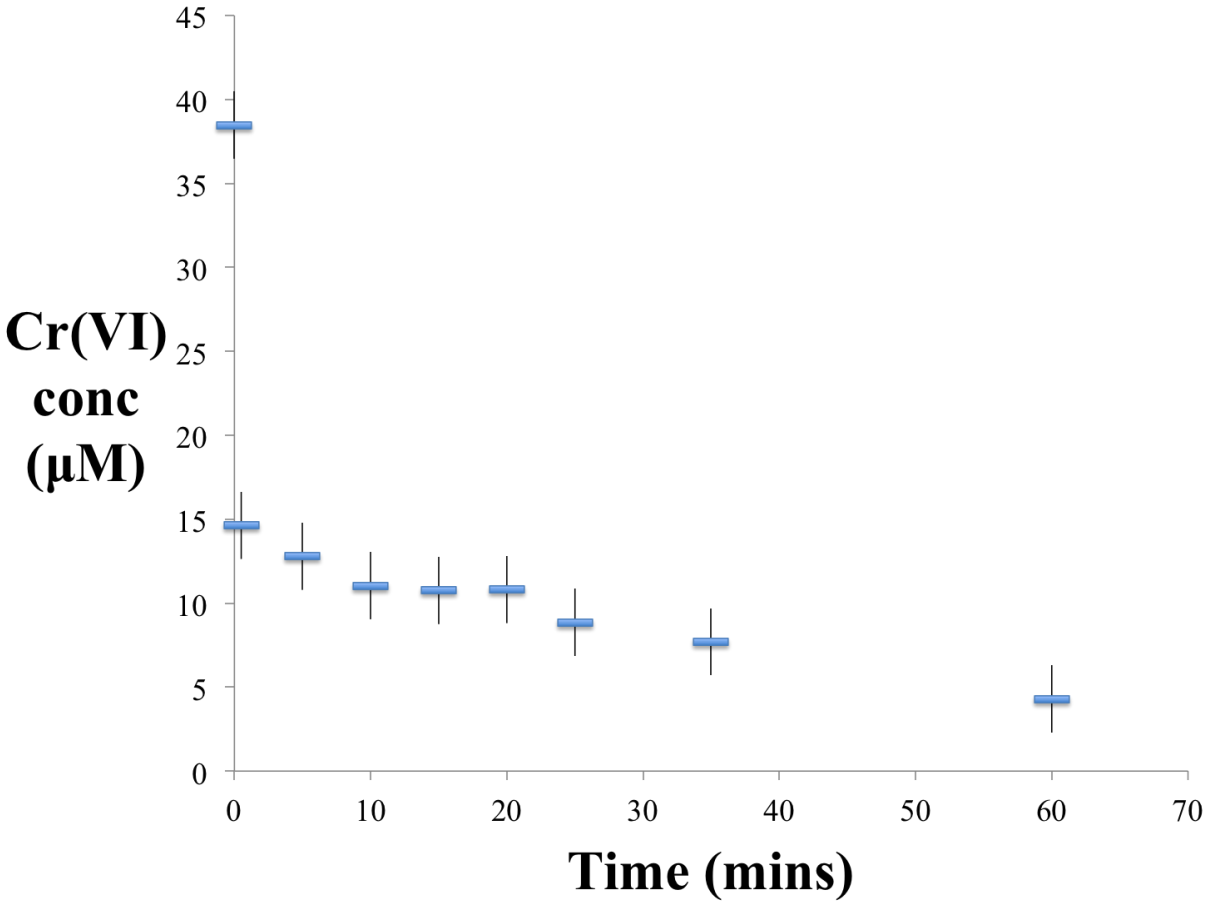

Figure 1. Cr(VI) concentrations of preliminary Cr(VI) experiment to establish kinetics; t=0 value is calculated from the amount of Cr(VI) injected. Initial ascorbate concentration was 115 µM.

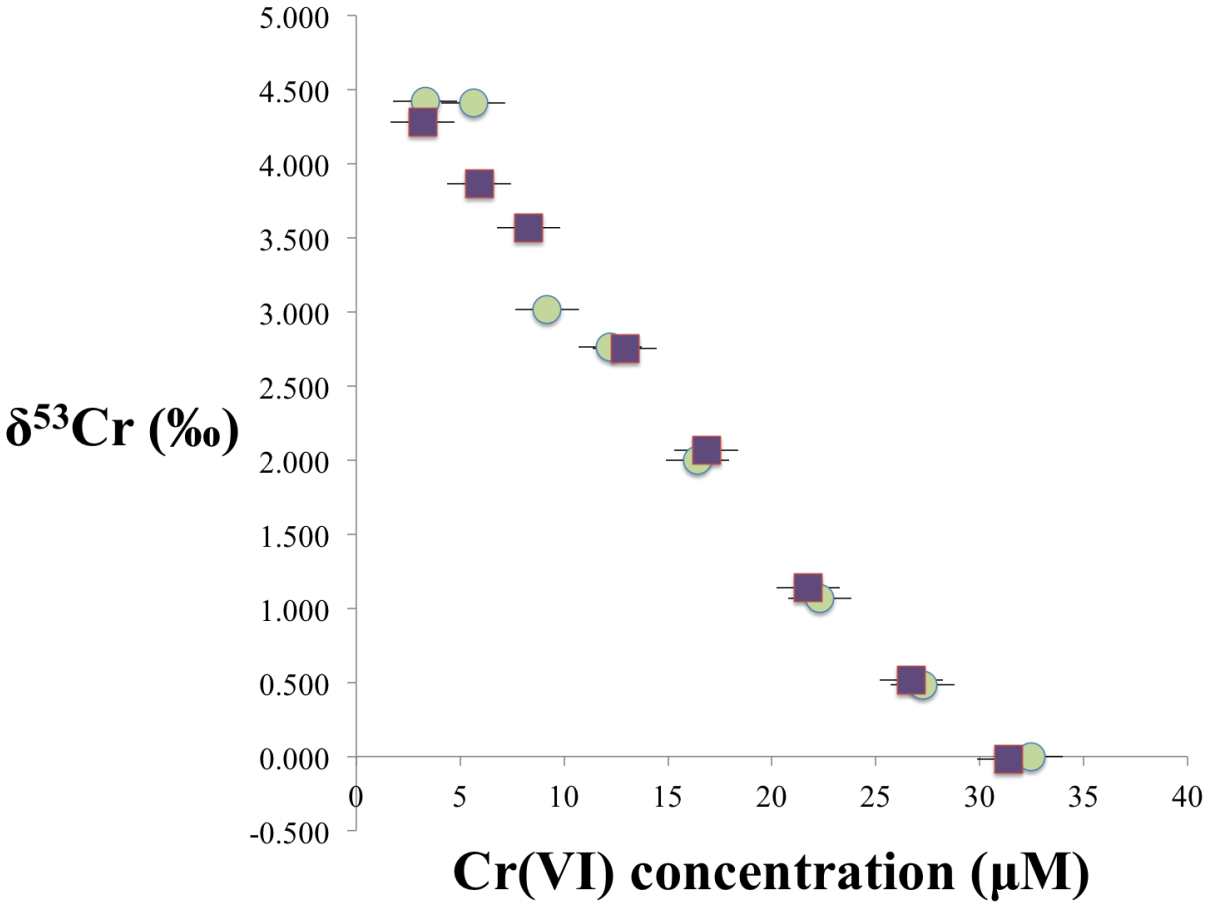

Figure 2. Concentration of Cr(VI) versus δ^{53}Cr of experiments A (circles) and B (squares) using step-wise ascorbate injection method.

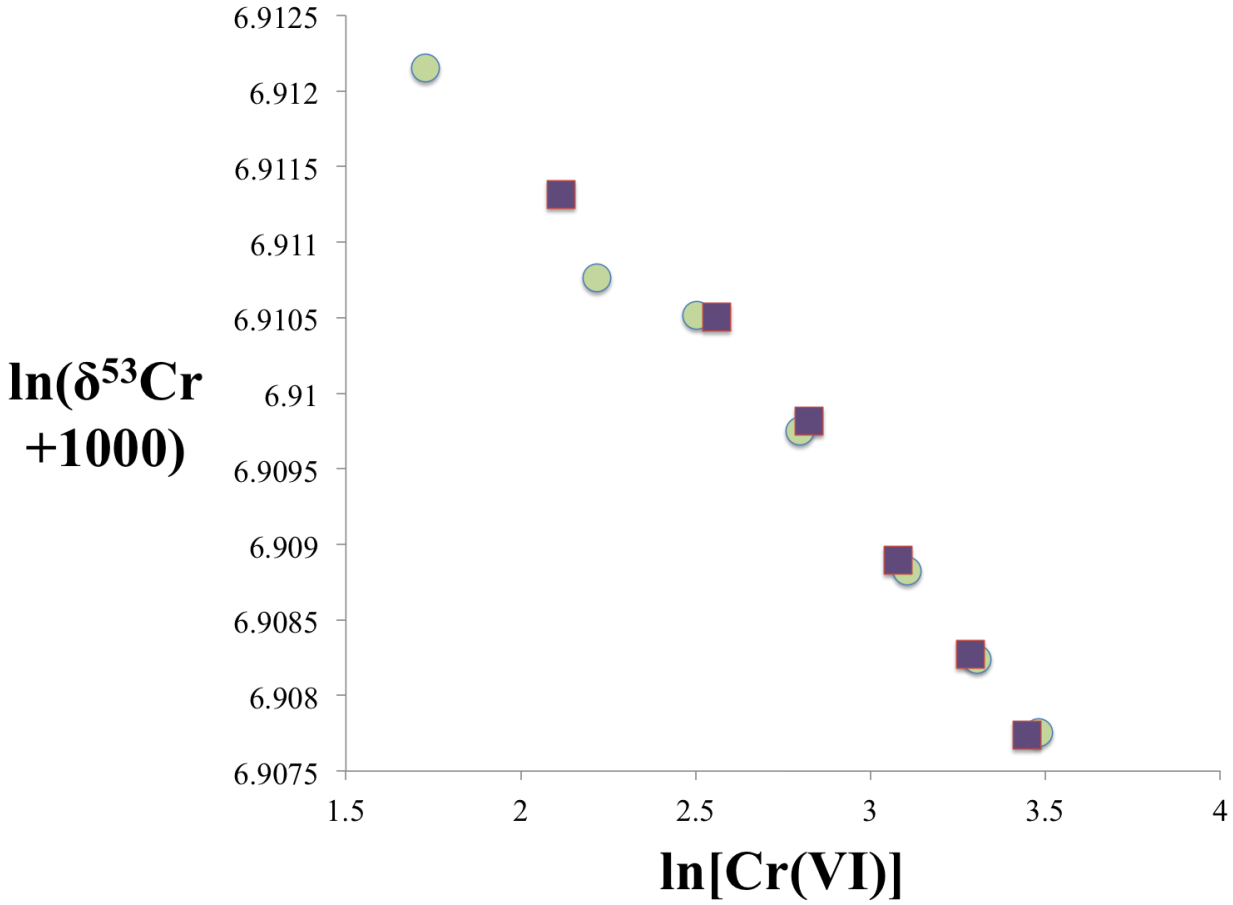

Figure 3. Plot of ln[Cr(VI)] versus ln(δ^{53}Cr + 1000‰) for experiments A (circles) and B (squares). Uncertainties given by the size of the data points.

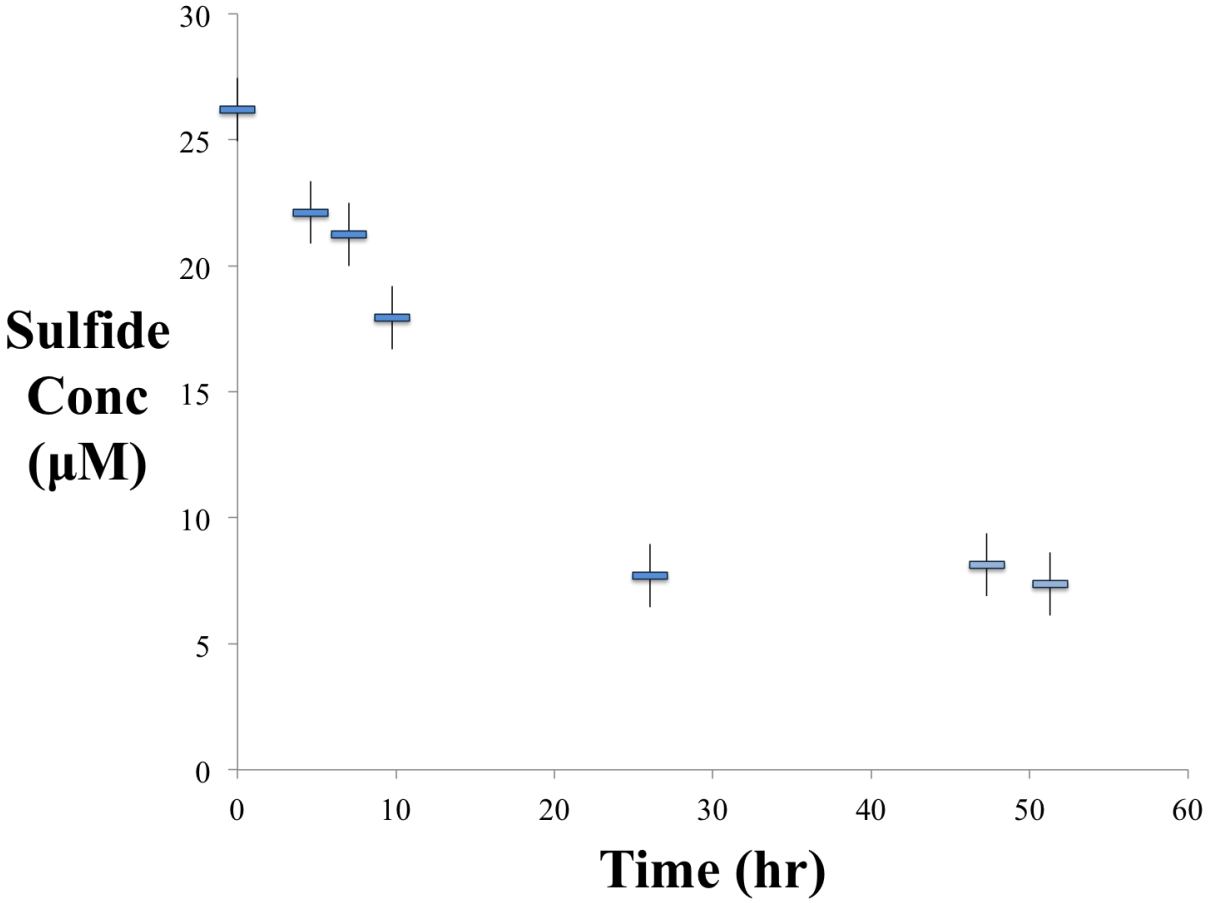

Figure 4. Concentrations of sulfide over time in a preliminary experiment that reveals reaction rate. Reduction was not so rapid as to cause diffusion-limitation effects before mixing was complete.

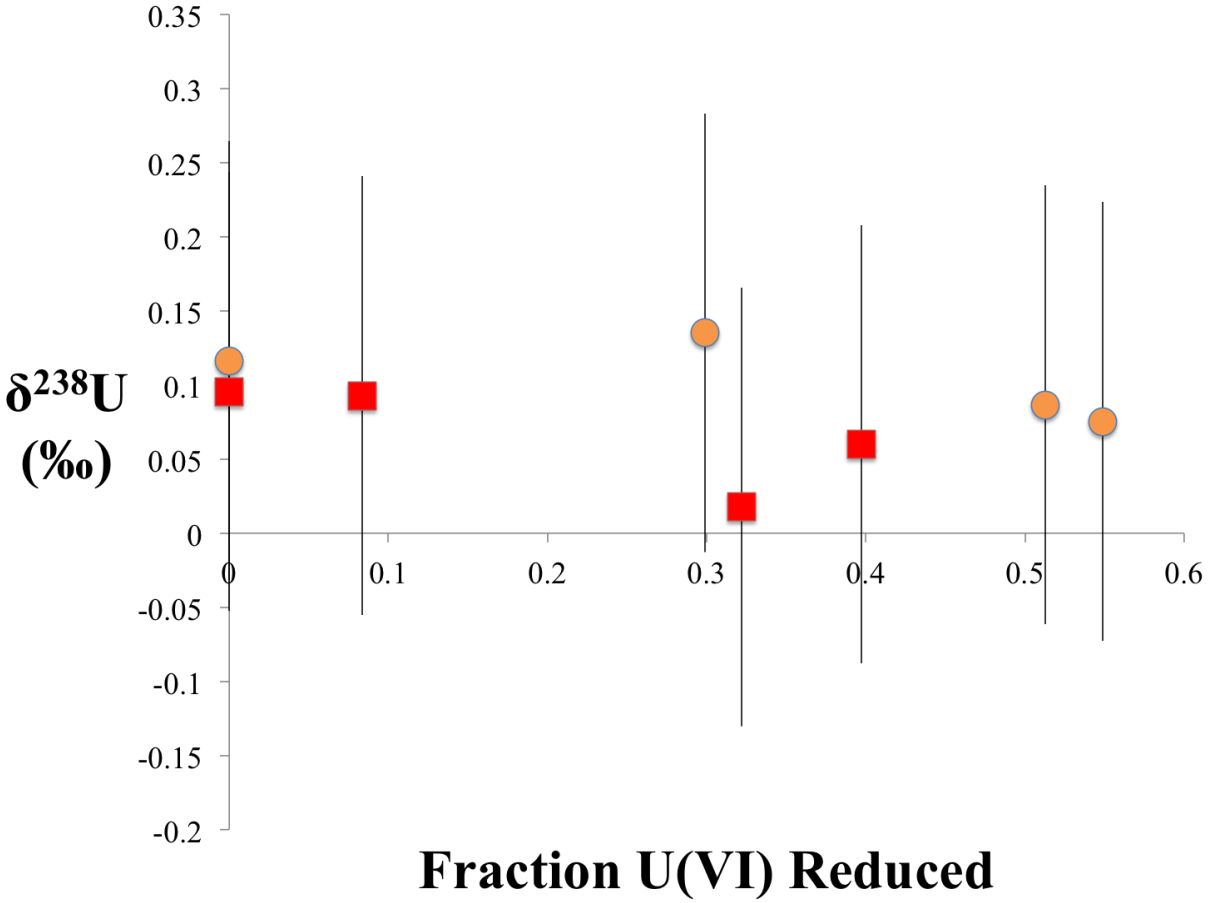

Figure 5. Fraction U(VI) reduced versus $\delta^{238}U$ for low U(VI) concentration experiments: experiment 1 (squares) and experiment 3 (circles).

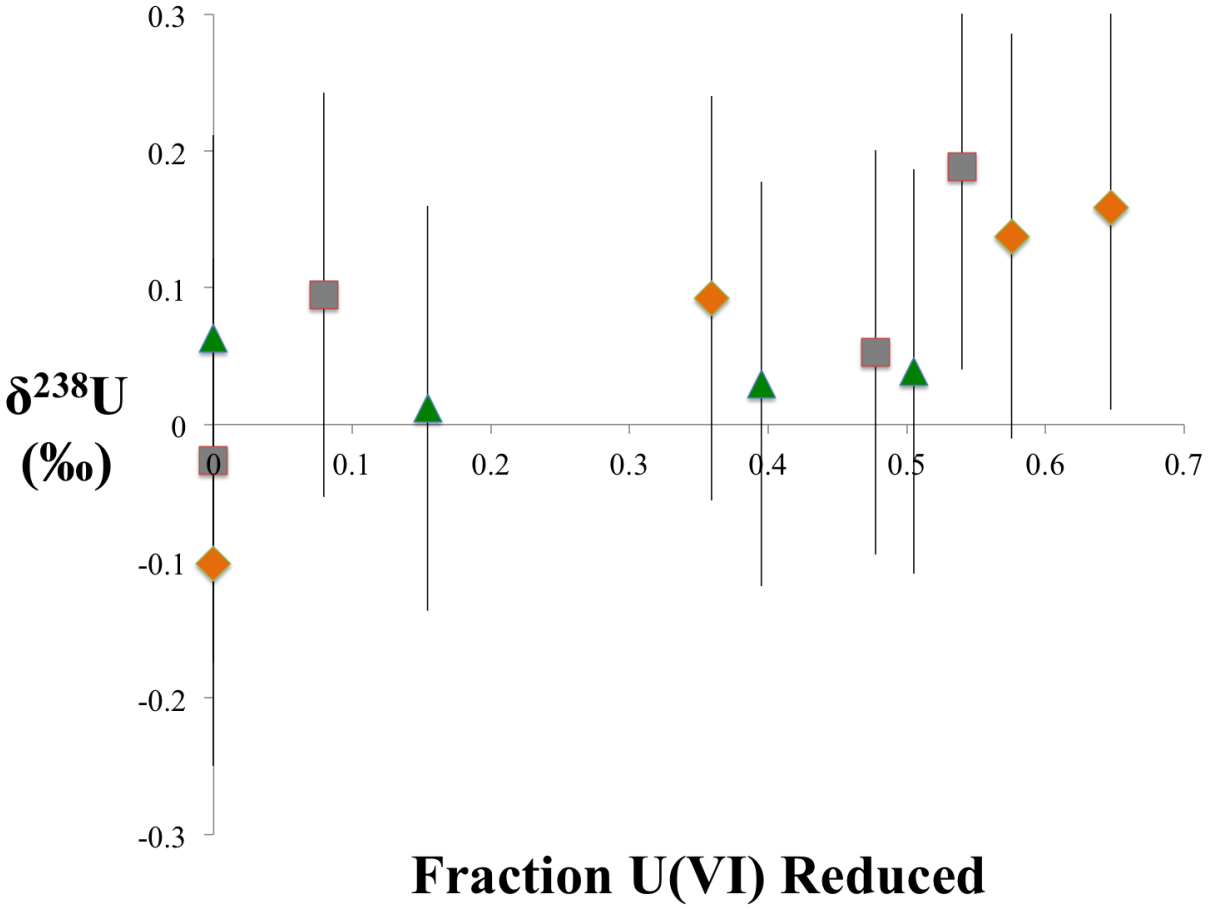

Figure 6. Fraction U(VI) reduced versus $\delta^{238}U$ for high concentration experiments: experiment 2 (diamonds), experiment 4 (squares) and experiment 5 (triangles)

Table 1. Concentration of Cr(VI) and pH of preliminary experiment

Time (min)	Concentration Cr(VI) (μM)	pH
0	38	---
0.5	15	6.64
5	13	6.69
10	11	6.70
15	11	6.74
20	11	6.74
25	9	6.77
35	8	6.80
60	4	6.72

Table 2. Cr(VI) concentrations and $\delta^{53}Cr$ for Cr(VI) reduction experiments.

Sample	Ascorbate conc per step before reaction (μM) [1]	Cumulative ascorbate added (μM)	Cr(VI) conc (μM)[2]	Cr(VI) conc (μM) [3]	$\delta^{53}Cr$ (‰)
Expt A					
step-0	0	0	31.6	32.5	0.00
step-1	11.6	11.6	26.6	27.3	0.48
step-2	11.9	23.6	22.2	22.3	1.07
step-3	12.3	35.2	16.5	16.4	2.00
step-4	12.6	48.4	11.9	12.2	2.76
step-5	13.1	61.5	8.1	9.2	3.02
step-6	13.7	75.2	5.0	5.6	4.41
step-7	15.1	90.4	2.5	3.3	4.42
Expt B					
step-0	0	0	31.1	31.4	-0.02
step-1	11.6	11.6	26.4	26.7	0.52
step-2	11.9	23.6	21.5	21.7	1.14
step-3	12.3	35.8	16.5	16.8	2.06
step-4	12.6	48.4	11.9	12.9	2.75
step-5	13.1	61.5	8.3	8.3	3.57
step-6	13.8	75.3	4.9	5.9	3.86
step-7	15.2	90.5	2.4	3.2	4.28

[1]determined using volume of ascorbate added and remaining experimental volume
[2] measured using colorimetric method
[3] measured using double spike isotope dilution method

Table 3. Sulfide concentrations after the first injection of sulfide in U(VI) reduction experiment 2

Time (hrs)	Sulfide conc (μM) [1]
0.00	25.2
4.50	21.7
7.00	21.2
9.50	18.0
26.00	7.9[2]
47.25	8.4[2]

[1] measured colorimetrically
[2] this value is less than the detection limit of the colorimetric method
Uncertainty in sulfide concentration is ±1.25 μM

Table 4. U(VI) concentrations, sulfide concentrations, and $\delta^{238}U$ for U(VI) reduction experiments.

Sample	Time (days)	Sulfide concentration before reaction [1] (μM)	Cumulative sulfide added (μM)	U(VI) concentration [2] (μM) +/-??	$\delta^{238}U$ (‰) +/-??
Expt 1					
1-a	0	12.1[3]	12.1	182.9	0.116
1-b	2.8	---	12.1	128.2	0.135
1-c	3.2	---	12.1	89.1	0.086
1-d	3.7	19.7[4]	31.8	82.5	0.075
Expt 2					
2-a	0	25.2[3]	25.2	334.2	0.101
2-b	2.1	---	25.2	214.2	0.092
2-c	2.5	---	25.2	141.9	0.138
2-d	3.0	27.5[4]	52.7	118.00	0.159
Expt 3					
3-a	0	11.6[3]	11.6	181.1	0.096
3-b	2.8	---	11.6	165.9	0.093
3-c	3.2	---	11.6	122.8	0.018
3-d	3.7	21.6[4]	33.2	109.1	0.060
Expt 4					
4-a	0	23.2[3]	23.2	324.6	0.027
4-b	2.0	---	23.2	298.8	0.095
4-c	2.4	---	23.2	169.6	0.053
4-d	2.9	43.1[4]	66.3	149.4	0.188
Expt 5					
5-a	0	25.7[3]	25.7	317.0	0.063
5-b	2.0	---	25.7	268.1	0.012
5-c	2.4	---	25.7	191.8	0.030
5-d	2.9	45.6[4]	71.3	156.9	0.039
Duplicate analyses					
2-c	2.5			141.8	0.092
3-a	0			181.0	0.050
1-d	3.7			82.5	0.047

[1] measured colorimetrically at time of sampling
[2] measured during isotope measurement using double spike isotope method
[3] aliquot of sulfide added after sample was removed
[4] aliquot of sulfide added before sample was removed
--- N/A